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Mesoderm Induction: A Postmodern View

Minireview

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The origin of all tissues in the adult animal can be traced back to one of three primary germ layers: endoderm (gut), mesoderm (muscle, bone, and connective tissues), and ectoderm (epidermis and neural tissue). Germ layer formation is one of the first subdivisions that occurs in embryonic development, and its regulation has engaged developmental biologists for over a century. The seminal work of Nieuwkoop (1969) demonstrated that a signal released by the most vegetal cells, the prospective endoderm, converts the overlying prospective ectoderm toward a mesodermal fate, creating the three germ layers of the amphibian embryo (Figure 1A; reviewed by Harland and Gerhart, 1997). Later experiments showed that the endogenous mesoderm-inducing signal is present as early as the 32-cell stage (Jones and Woodland, 1987), many hours before transcription occurs in the embryo, and is mimicked in vitro by members of the TGF-β and FGF families of secreted growth factors. It is now clear that mesoderm induction requires a TGF-β signal operating in concert with an FGF signal. (TGF-β is used throughout this review to connote any member of the TGF-β superfamily.) Endoderm development also requires TGF-β signaling, suggesting that mesoderm and endoderm may be induced by a common pathway (Henry et al., 1996). However, it is not at all clear how mesoderm and endoderm induction are spatially separated in the embryo.

The widely accepted synthesis of these data has been that the endogenous mesoderm-inducing signal must be present as a maternal mRNA or protein encoding a secreted factor, most likely a TGF-β family member, that is localized to the vegetal cytoplasm during oogenesis. Fortunately, Melton and colleagues identified a maternal, vegetally localized mRNA encoding the TGF-β family member *Vg1* (Weeks and Melton, 1987). The *active* form of Vg1 protein, however, has never been detected in vivo, and ectopically expressed wild-type *Vg1* does not induce mesoderm or endoderm. Advocates for *Vg1* speculate that its processing must be tightly regulated, and that the active form is present at undetectable levels in vivo. In support of a role for *Vg1*, a recent study using a dominant-negative mutant indicates that *Vg1* is required for the development of dorsal mesoderm and dorsal endoderm (Joseph and Melton, 1998). However, the dominant-negative *Vg1* had no effect on ventral and lateral mesoderm (ventral and lateral endoderm were not examined), arguing that a different TGF-β family member is involved in mesoderm, and possibly endoderm, induction. Several authors have presented evidence opposing and favoring a role for activin in early *Xenopus* development, and it still remains possible that the early embryo contains a yet undiscovered member of the TGF-β family.

And the Mesoderm-Inducing Signal Is ... a Transcription Factor?

The work of Zhang et al. (1998) in this issue of *Cell* seriously challenges the orthodox view of mesoderm induction. Their work shows that a crucial component of the vegetal maternal mesoderm-inducing signal is not a secreted factor, but a member of the intriguing T-box transcription factor family. Since transcription factors cannot act until the start of zygotic transcription at the mid-blastula (4000-cell) stage, a clear implication of this work is that mesoderm (and endoderm) induction occurs much later than originally supposed.

Over the last two years, four groups have described a novel *Xenopus* transcription factor containing a T-box DNA-binding motif and gave it a variety of names including *VegT*, *Xombi*, *Antipodean*, and *Brat* (citations can be found in Zhang et al., 1998). *VegT* is first observed as a maternal transcript localized to the vegetal hemisphere of eggs and embryos, which corresponds primarily to the prospective endoderm and possibly some of the mesoderm (Figure 1B), in a pattern quite similar to that of *Vg1* (Weeks and Melton, 1987). Just before gastrulation, zygotic *VegT* transcripts are found throughout the mesoderm (Figure 1C). Ectopic expression experiments by all of the groups indicated an important role for *VegT* in regulating mesoderm and endoderm specification and morphogenesis, but it was not possible to distinguish between the maternal and zygotic roles of *VegT*.

Zhang et al. (1998) used antisense oligonucleotides to specifically deplete the maternal *VegT* mRNA and

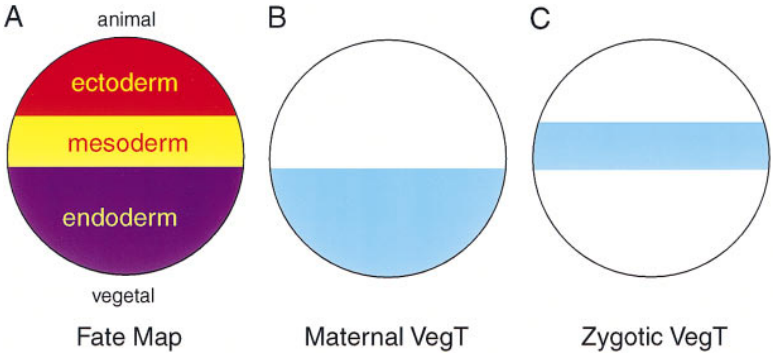

Figure 1. *Xenopus* Fate Map and *VegT* Expression

(A) Fate map of a *Xenopus* embryo prior to gastrulation.
(B) Maternal *VegT* expression is confined to the vegetal hemisphere.
(C) Zygotic *VegT* expression just after the start of gastrulation is throughout the prospective mesoderm.

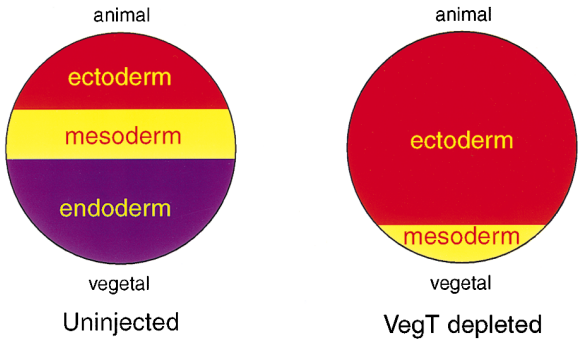

Figure 2. Shift of Germ Layer Fate Map due to *VegT*-Depletion, as Proposed by Zhang et al. (1998)

See text for details.

found two very surprising results. First, *VegT*-depleted embryos did not form endoderm, and there was a concomitant vegetal shift in the fate map such that mesoderm formed mainly from the vegetal pole, and ectoderm formed from equatorial and vegetal cells (Figure 2). This is strikingly similar to what happens when TGF-β signaling is blocked in *Xenopus* embryos with a truncated TGF-β receptor; mesoderm is lost from the equator and the vegetal pole develops as mesoderm and ectoderm, not as endoderm (Hemmati-Brivanlou and Melton, 1992; Henry et al., 1996). These results suggest a common connection between the role of *VegT* and mesoderm-inducing TGF-β signals. Second, as originally described by Nieuwkoop (1969), explants of vegetal pole tissue can induce explants of prospective ectoderm (the "animal cap") to form mesoderm. When these experiments were repeated by Zhang et al. (1998), it was found that *VegT*-depleted vegetal tissue does not secrete a mesoderm-inducing signal, although very weak induction of the mesodermal marker *Xbra* was still observed. However, *VegT*-depleted animal caps could still be induced to form mesoderm by untreated vegetal tissue, demonstrating that *VegT* is essential for the release of the mesoderm-inducing signal but is not required to receive it.

When Is Mesoderm Induced?

The idea that *VegT* activates the mesoderm-inducing signaling at the mid-blastula stage is hard to reconcile with the demonstration that mesoderm can be induced at the 32-cell stage (Jones and Woodland, 1987). One possibility is that mesoderm induction is biphasic, consisting of a weak maternal signal and a strong *VegT*-dependent signal activated at the mid-blastula stage. This is consistent with a previous report that the major mesoderm-inducing signal was released at the onset of transcription, but that a much less effective signal was present earlier (Wylie et al., 1996). A major challenge now lies in identifying the targets of the maternal VegT protein. *VegT* might activate the transcription of a secreted factor that either supersedes the maternal signal, or acts synergistically with it. Alternatively, *VegT* might activate the transcription of a protein involved in the processing or release of a maternal signal. While it might be tempting to speculate that *VegT* activates the processing of the Vg1 preprotein, the experimental evidence that *Vg1* is only involved in dorsal mesoderm and endoderm development (Joseph and Melton, 1998) suggests that this may not be the case.

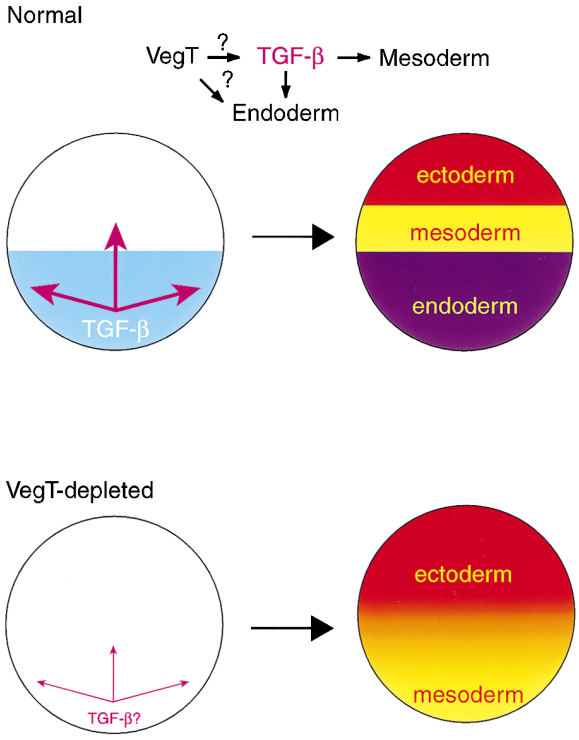

Figure 3. Possible Model for Maternal *VegT* Function

In normal embryos, *VegT* (shown in light blue) promotes endodermal fate and the activation of a TGF-β signal (purple arrows). The TGF-β signal is not only necessary for endoderm development, but is also a potent mesoderm-inducing factor that induces mesoderm from prospective ectodermal cells at the equator. When *VegT* is depleted, a low-level maternal signal induces mesoderm in the vegetal part of the embryo; we suggest it also weakly induces mesoderm at the equator. Endoderm is not formed either due to insufficient levels of TGF-β signaling, or loss of an autonomous function of *VegT*.

The Role of VegT in Early Patterning

It is difficult to arrive at a satisfactory model that reconciles all of the recent results with the large collection of previous observations. The simplest model is that VegT protein has a graded distribution and activates a morphogenetic gradient of TGF-β signaling (Figure 8, model 3 in Zhang et al., 1998). In support of this, ectopic expression of *VegT* in animal caps induces mesoderm and endoderm, with endoderm induced at higher doses of *VegT* (Horb and Thomsen, 1997). Although this model is very seductive for developmental biologists reared on the gradient models of Wolpert (1969), increasing levels of TGF-β signaling do not induce endodermal genes separately from mesodermal genes (Henry et al., 1996). Moreover, analysis of TGF-β signaling in vivo using a TGF-β-responsive promoter to drive expression of a reporter gene did not reveal any evidence for a TGF-β gradient (Watabe et al., 1995). Finally, while nothing is yet known about the distribution of the VegT protein, the mRNA appears to be uniformly distributed.

Our favored interpretation is a variant of model 1 as proposed by Zhang et al. (1998: Figure 8), as follows. The vegetal pole of the egg contains a weak mesoderm-inducing signal of unknown identity, as well as *VegT*

mRNA. At the onset of transcription, the maternal VegT promotes endodermal fate and activates high levels of TGF-β signaling; these two effects may be related (Figure 3). The high levels of TGF-β signaling act as a strong mesoderm-inducing signal and induce mesoderm in overlying prospective ectoderm at the equator of the embryo, establishing the three germ layers of the embryo. In the absence of *VegT*, the low-level maternal signal induces mesoderm in the vegetal pole and only weakly at the equator (Figure 3); this is the signal detected in earlier experiments (Jones and Woodland, 1987; Wylie et al., 1996). Consistent with this, Zhang et al. (1998) observed very weak expression of the mesodermal marker *Xbra* in the marginal zone of *VegT*-depleted embryos and in animal caps conjugated with *VegT*-depleted vegetal tissue.

The work of Zhang et al. (1998) opens up as many questions as it answers. Where exactly is VegT protein located with respect to the fate map of the embryo? Does VegT activate transcription of a TGF-β or does it process or release an existing signal? What is the role of the maternal intercellular signal? Does VegT act synergistically with the maternal signal? Is the maternal signal required for the VegT-dependent signal to be effective? Finally, the precise role of VegT in endoderm formation is unclear. Is the primary role of VegT to specify endoderm, which secondarily releases inducing signals, or does endoderm form in response to inducing signals activated by VegT in the vegetal pole?

Role of VegT in Mesodermal Patterning

An earlier study addressed the function of *VegT* by conjugating the DNA-binding domain of VegT to the repressor domain of *Drosophila engrailed*. Ectopic expression of this construct inhibited the formation of mesoderm. Given the important function of the maternal *VegT* described by Zhang et al. (1998), this effect could be explained solely in terms of blocking the maternal function of *VegT*. What then is the function of the zygotic *VegT* expression in the mesoderm? Recent studies of the *VegT* ortholog in zebrafish indicate that the zygotic expression of *VegT* is likely to have as critical a role in regulating mesodermal and endodermal patterning and morphogenesis as its maternal expression has in mesoderm and endoderm formation.

The zebrafish ortholog of *VegT* was recently identified and shown to be the *spadetail* gene (Griffin et al., 1998). The description of the *spadetail* mutant phenotype revealed unexpected complexity in the control of trunk and tail formation in zebrafish, reminiscent of the gap gene phenotypes in *Drosophila*. *Spadetail* mutant embryos have a profound deficit in mesodermal and endodermal derivatives that is restricted to the trunk region, whereas tail and notochord development are relatively normal (Kimmel et al., 1989). In addition, morphogenesis of prospective trunk mesodermal cells is deranged such that these cells end up in a disorganized mass at the tip of the tail (Ho and Kane, 1990). Thus, *spadetail* plays a critical role in controlling both the fate and morphogenesis of mesoderm and endoderm in the zebrafish trunk.

Although *spadetail* expression is very similar to the zygotic expression of *VegT*, a significant difference is that *spadetail* is not expressed maternally. Furthermore, mesoderm and endoderm induction appear to be unaffected by the loss of *spadetail* function, raising the possibility that the initial events in mesoderm and endoderm induction in *Xenopus* are not conserved in other vertebrates. An alternative possibility is that the partial genome duplication that occurred during the evolution of fish resulted in two *VegT*-like genes, *spadetail* and an as yet unidentified T-box gene, which then became subspecialized with respect to the maternal and zygotic functions of *VegT*. The resolution of this issue will provide important insights into the basic mechanisms driving germ layer specification in vertebrate embryos.

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